CLAIMS:

The following is a listing of all claims in the application with their status and the text of all active claims.

1. **(CURRENTLY AMENDED)** A DNA construct having a formula

pY – SP – B(1-29)-A(1-21),

where A) pY is any promoter in yeast, B) SP encodes a signal peptide region that enables the secretion of polypeptides expressed in yeasts, and is derived from either *Schwanniomyces occidentalis* glucoamylase signal peptide sequence or from *Carcinus maenas* crustacean hyperglycemic ~~harmone~~ hormone signal peptide sequence, and lies to the N-terminus of the insulin peptide region B(1-29)-A(1-21) and C) B(1-29)-A(1-21) encodes, upon expression, the insulin peptide region in which B(1-29) is the B chain of insulin from amino acid 1 to amino acid 29, A(1-21) is the A chain of insulin from amino acid 1 to amino acid 21, and that the amino acid 29 of the B chain directly connects, by means of a peptide bond, the amino acid 1 of the A chain and the expression of SP – B(1-29)-A(1-21) region is under the control of the promoter - pY.

2. **(ORIGINAL)** A DNA construct according to claim 1 where the SP is derived from *Schwanniomyces occidentalis* glucoamylase signal peptide sequence.

3. **(CURRENTLY AMENDED)** A DNA construct according to claim 1 where the SP is derived from *Carcinus maenas* crustacean hyperglycemic ~~harmone~~ hormone signal peptide sequence.

4. **(ORIGINAL)** A DNA construct according to claim 2 in which the SP carries a kex protease cleavage site.

5. **(ORIGINAL)** A DNA construct according to claim 3 in which the SP carries a kex protease cleavage site.

6. **(ORIGINAL)** A DNA construct according to claim 2 in which the SP does not carry any kex protease cleavage site.

7. **(ORIGINAL)** A DNA construct according to claim 3 in which the SP does not carry any kex protease cleavage site.

8. **(ORIGINAL)** A DNA construct according to claim 6 in which the SP has a single methionine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

9. **(ORIGINAL)** A DNA construct according to claim 7 in which the SP has a single methionine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

10. **(ORIGINAL)** A DNA construct according to claim 6 in which the SP has either a single Arginine or a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

11. **(ORIGINAL)** A DNA construct according to claim 7 in which the SP has either a single Arginine or a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

12. **(WITHDRAWN)** A polypeptide SP-B(1-29)-A(1-21) B(1-29)-A(1-21), where SP is a signal peptide region that enables the secretion of polypeptides expressed in yeasts and is derived from either Schwanniomyces occidentalis glucoamylase signal peptide sequence or from Carcinus maenas crustacean hyperglycemic harmone signal peptide sequence, and lies to the N-terminus of the insulin peptide region B(1-29)-A(1-21), and further where B(1-29) is the B chain of insulin from amino acid 1 to amino acid 29, A(1-21) is the A chain of insulin from amino acid 1 to amino acid 21, and the amino acid 29

of the B chain directly connects, by means of a peptide bond, the amino acid 1 of the A Chain.

13. **(WITHDRAWN)** A polypeptide according to claim 12 where the SP is derived from Schwanniomyces occidentalis glucoamylase signal peptide sequence.

14. **(WITHDRAWN)** A polypeptide according to claim 12 where the SP is derived from Carcinus maenas crustacean hyperglycemic harmone signal peptide sequence.

15. **(WITHDRAWN)** A polypeptide according to claim 13 in which the SP carries a kex protease cleavage site.

16. **(WITHDRAWN)** A polypeptide according to claim 14 in which the SP carries a kex protease cleavage site.

17. **(WITHDRAWN)** A polypeptide according to claim 13 in which the SP does not carry any kex protease cleavage site.

18. **(WITHDRAWN)** A polypeptide according to claim 14 in which the SP does not carry any kex protease cleavage site.

19. **(WITHDRAWN)** A polypeptide according to claim 17 in which the SP has a single methionine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

20. **(WITHDRAWN)** A polypeptide according to claim 18 in which the SP has a single methionine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

21. **(WITHDRAWN)** A polypeptide according to claim 17 in which the SP has either

a single Arginine or a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

22. **(WITHDRAWN)** A polypeptide according to claim 18 in which the SP has either a single Arginine or a single Lysine residue placed such that it is just adjacent and N-terminus to the polypeptide encoded by the insulin peptide region B(1-29)-A(1-21).

23. **(ORIGINAL)** A DNA construct according to claim 1 in which the promoter, pY, is of yeast origin.

24. **(ORIGINAL)** A DNA construct according to claim 23 in which the promoter, pY, is either the methanol oxidase promoter (MOX-P) or Formaldehyde dehydrogenase promoter (FMDH-P) or Formate dehydrogenase promoter (FMD-P) or Dihydroxyacetone synthase promoter (DHAS-P).

25. **(ORIGINAL)** A process for the expression of insulin in yeasts which consists of transforming the said yeast with a plasmid that carries the DNA construct of claim 1, culturing the said transformed yeasts in an appropriate culture and isolating the insulin containing polypeptide from the culture medium.

26. A process according to claim 25 where the yeast is selected from genera Hansenula, Saccharomyces, Pichia, Kluyveromyces.

27. **(ORIGINAL)** A process according to claim 26 where the yeast is *Hansenula polymorpha.*

28. **(ORIGINAL)** A DNA construct of claim 1 in which B(1-29) is the B chain of human insulin from amino acid 1 to amino acid 29, A(1-21) is the A chain of human insulin from amino acid 1 to amino acid 21.

29. **(WITHDRAWN)** Process for the isolation, purification and conversion to native insulin, of the polypeptides of claims 15 consisting of the following steps:
a) Clarification of the culture supernatants containing the above polypeptides.

b) Subjecting the clarified culture supernatants to cation exchange chromatography.

c) Isoelectric precipitation of the cation exchange chromatography derived polypeptides.

d) Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.

e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase chromatography.

f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.

g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was purified on a reverse phase HPLC column.

h) Isoelectric precipitation of the purified insulin.

30. **(WITHDRAWN)** A process according to claim 29 where any two steps are performed in sequence.

31. **(WITHDRAWN)** Process for the isolation, purification and conversion to native insulin, of the polypeptides of claim 16 consisting of the following steps:

a) Clarification of the culture supernatants containing the above polypeptides.

b) Subjecting the clarified culture supernatants to cation exchange chromatography.

c) Isoelectric precipitation of the cation exchange chromatography derived polypeptides.

d) Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.

e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase chromatography.

f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.

g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was purified on a reverse phase HPLC column.

h) Isoelectric precipitation of the purified insulin.

32. **(WITHDRAWN)** A process according to claim 31 where any two steps are performed in sequence.

33. **(WITHDRAWN)** Process for the isolation, purification and conversion to native insulin, of the polypeptides of claim 21 consisting of the following steps:
a) Clarification of the culture supernatants containing the above polypeptides.

b) Subjecting the clarified culture supernatants to cation exchange chromatography.

c) Isoelectric precipitation of the cation exchange chromatography derived polypeptides.

d) Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.

e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase chromatography.

f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.

g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was purified on a reverse phase HPLC column.

h) Isoelectric precipitation of the purified insulin.

34. **(WITHDRAWN)** A process according to claim 33 where any two steps are performed in sequence.

35. **(WITHDRAWN)** Process for the isolation, purification and conversion to native insulin, of the polypeptides of claim 22 consisting of the following steps:
a) Clarification of the culture supernatents containing the above secreted polypeptides.

b) Subjecting the clarified culture supernatents to cation exchange chromatography.

c) Isoelectric precipitation of the cation exchange chromatography derived polypeptides.

d) Transpeptidation reaction in which the polypeptide precipitates were converted to insulin-t-butyl ester-t-butyl ether.

e) Purification of the insulin-t-butyl ester-t-butyl ether, by reverse phase chromatography.

f) Hydrolysis of the insulin-t-butyl ester-t-butyl ether to native insulin.

g) Purification of insulin wherein the insulin obtained from the hydrolysis reaction was purified on a reverse phase HPLC column.

h) Isoelectric precipitation of the purified insulin.

36. **(WITHDRAWN)** A process according to claim 35 where any two steps are performed in sequence.